

REVIEW ARTICLE

Applications of Single-Cell Omics Technologies for Induced Pluripotent Stem Cell-Based Cardiovascular Research

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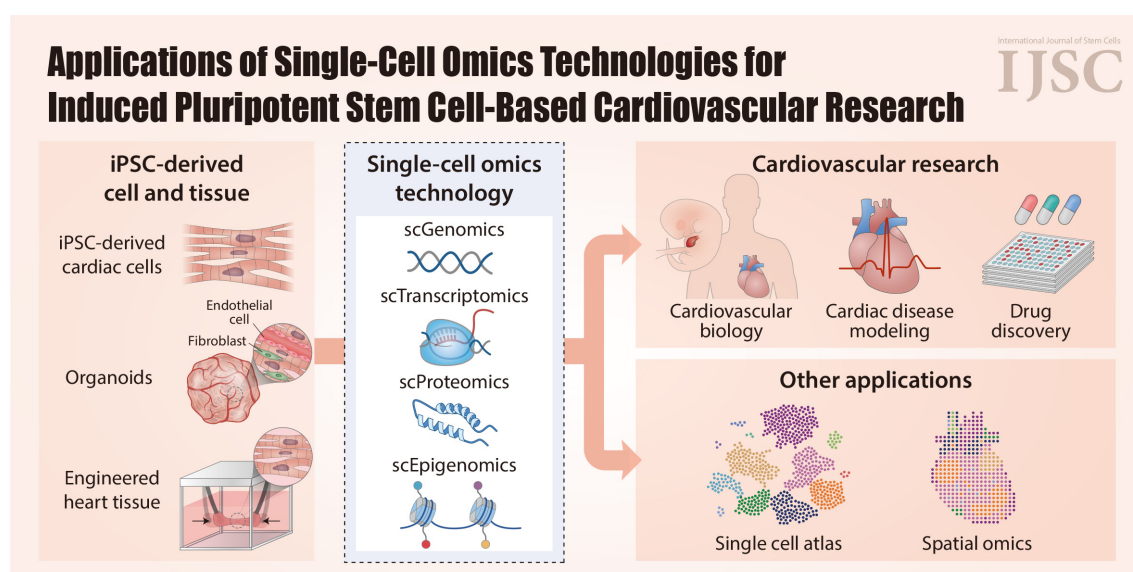
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Single-cell omics technologies have transformed our investigation of genomic, transcriptomic, and proteomic landscapes at the individual cell level. In particular, the application of single-cell RNA sequencing has unveiled the complex transcriptional variations inherent in cardiac cells, offering valuable perspectives into their dynamics. This review focuses on the integration of single-cell omics with induced pluripotent stem cells (iPSCs) in the context of cardiovascular research, offering a unique avenue to deepen our understanding of cardiac biology. By synthesizing insights from various single-cell technologies, we aim to elucidate the molecular intricacies of heart health and diseases. Beyond current methodologies, we explore the potential of emerging paradigms such as single-cell/spatial omics, delving into their capacity to reveal the spatial organization of cellular components within cardiac tissues. Furthermore, we anticipate their transformative role in shaping the future of cardiovascular research. This review aims to contribute to the advancement of knowledge in the field, offering a comprehensive perspective on the synergistic potential of transcriptomic analyses, iPSC applications, and the evolving frontier of spatial omics.

Keywords: Transcriptome analysis, Induced pluripotent stem cells, Organoids, Cardiovascular diseases

Introduction

Recent progress in single-cell technologies has opened up new avenues for profiling biological macromolecules with an unparalleled level of precision, allowing for a deeper understanding of cellular diversity across various contexts (1). Single-cell omics is the profiling of individual cells obtained from a population in different cellular states, where the heterogeneous landscape of both the steady-state and pathological conditions can be dissected at a single-cell resolution. The molecular term “omics” means a comprehensive or global assessment of a set of molecules, capturing the full scope of the molecular information (2).

The advancement in multidimensional genomic and

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proteogenomic analysis has also played a substantial role in enhancing our present understanding of cellular processes. This advantage has, in turn, catalyzed the progress of single-cell multi-omics studies. Since cell types and states can be inferred from RNA transcripts, sequencing RNA at single-cell resolution gave rise to a new era of transcriptomics. Compared to conventional bulk RNA sequencing, which inevitably masks the individual gene expressions, single-cell RNA sequencing (scRNA-seq) can identify discrete cell subtypes with high throughput and resolution. Due to its potential for integrative analyses, the field of scRNA-seq technology has been rapidly growing and getting increasingly recognized since the first publication in 2009 (2).

Consequently, single-cell-level approaches have particularly transformed the field of cardiac research. A common approach to studying cardiac development, physiology, and disease mechanisms (3) involves using human-induced pluripotent stem cells (iPSCs). By modeling cardiac tissues, iPSC-derived cardiac cells provide efficient structural, electrophysical, mechanical, and metabolic readouts. While various challenges remain, single-cell techniques coupled with omics analyses and iPSC technologies have been employed to study heterogeneity, cell-cell interactions (4), cellular lineages (5), and stem cell differentiation and maturation (6). This review elucidates the progression of single-cell omics technologies, focusing on single-cell transcriptomics and its pivotal application in cardiovascular research driven by iPSCs. Further, we discuss insights gained from integrating single-cell sequencing and iPSC technology in cardiac studies, particularly concerning cellular and molecular heterogeneity. Finally, we offer a broader perspective on the recent development of single-cell sequencing methodologies and their practical application in the wider sphere of cardiac research (Fig. 1).

Single-Cell Omics Technologies

Experimental procedures in single-cell omics, particularly those involving scRNA-seq, typically start with isolation of single-cell, subsequently followed by sequencing and downstream analyses (7). In the past, integrated fluidic circuit (IFC) systems like Fluidigm C1 were utilized for isolating cells into chambers, where they underwent lysis and reverse transcription (plate-based method). Fluorescence-activated cell sorting (FACS) replaced IFC by isolating cells and distinguishing live cells through viability stains (8). Plate-based systems have limitations in cell analysis, leading to the development of droplet-based methods. Here, single cells are encapsulated, undergo barcoding, and

reverse transcription, significantly improving throughput to analyze up to 10,000 cells with reduced read depth (9).

Both IFC systems and microfluidic devices in plate-based and droplet-based methods, respectively, restrict cell size processing to $< \sim 40 \mu\text{m}$, suitable for non-cardiomyocytes (CMs), neonate cardiomyocytes, and pluripotent stem cell-derived CMs. However, adult CMs ($> 100 \mu\text{m}$) are incompatible. Commercial FACS nozzles ($70 \sim 130 \mu\text{m}$) may damage live CMs. Successful large-particle FACS isolation of single CMs ($500 \mu\text{m}$ nozzle) has been reported (7). Most scRNA-seq studies in CMs rely on manual pick-up or the iCELL8 system (10). Alternatively, single nuclei of CMs, smaller and compatible with FACS and droplet-based systems, can be isolated and sequenced (11). Single-nucleus RNA sequencing (snRNA-seq), applicable to archived frozen specimens, minimizes gene expression alterations caused by dissociation (12). Comparing snRNA-seq and scRNA-seq in iPSC differentiation to CMs, scRNA-seq captures more genes (mitochondrial, ribosomal), while snRNA-seq detects more non-coding RNA and intronic region reads, considering the impact of CM multinucleation and polyploidy (13-15).

Integrating single-cell transcriptomics with single-cell epigenomics and single-cell proteomics, especially employing methods like single-cell Assay for Transposase-Accessible Chromatin-sequencing (scATAC-seq), serves to advance our understanding of cell clustering and gene expression interactions (16, 17). Spatial transcriptomics, employing multiplexed RNA imaging (e.g., seqFISH) or spatial barcoding (e.g., Visium, slide-seq), is advancing due to the complex, heterogeneous heart tissues, offering insights into tissue architectures and intercellular communications in the developing, normal, and diseased heart (4, 18). The subsequent sections of this review focus on recent cardiovascular research utilizing single-cell omics technologies.

Single-Cell Transcriptomics

Single-cell transcriptomics, particularly scRNA-seq, has revolutionized cardiac research by enabling detailed transcriptional profiling at the individual cell level. A typical scRNA-seq experiment involves several core steps: cell dissociation, single-cell capture, reverse transcription, amplification, library preparation, sequencing, and subsequent data analysis (19). Over time, diverse sequencing protocols have evolved, each tailored to specific aspects of transcriptomics since the publication of the first single-cell transcriptome profiling study in 2009 (20). These protocols are generally classified into three types based on cell-sorting processes and the location of cell barcoding (13): cell-per-

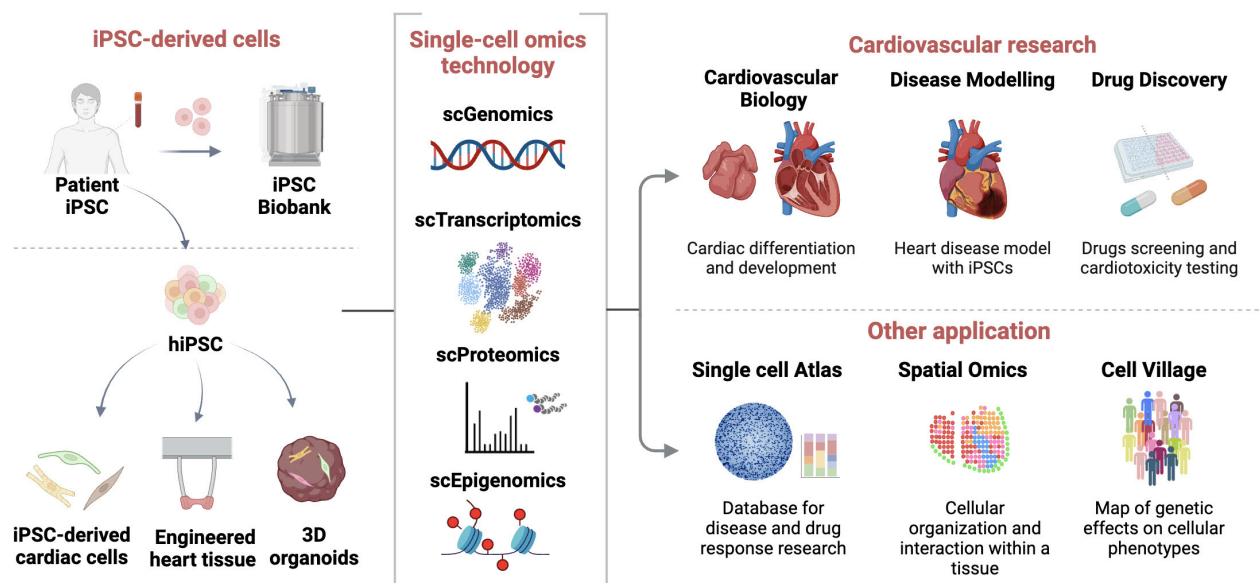


Fig. 1. Overview of the application of human induced pluripotent stem cells (hiPSCs) in single-cell omics technology and cardiovascular research.

well (CPW), droplet-based, and single-cell combinatorial indexing strategies (21).

The CPW method, an early strategy in single-cell sorting, places each cell into a well or tube, with one cell intentionally picked into individual tubes (20). Meanwhile, droplet-based techniques, like Drop-seq, inDrops, and 10X Genomics, use a co-flow system to encapsulate cells and barcoded microparticles within small droplets for mRNA capture. Recent advancements in droplet strategies, such as Drop-seq (14), inDrops (15), and 10X Genomics, have employed distinct bead-based approaches to further enhance single-cell transcriptome profiling. Single-cell combinatorial index-based methods, such as sci-RNA-seq and SPLiT-seq, represent a novel high-throughput strategy, treating intact cells or nuclei as individual units for indexing, with each unit uniquely labeled multiple times after pooling and splitting.

Furthermore, snRNA-seq has been introduced to complement scRNA-seq, especially in complex tissues like the heart, where isolating intact cells can be challenging (18, 22). For instance, Simonson et al. (23) have exploited snRNA-seq to map the transcriptional landscape of angiogenic endothelial cells in capillaries of end-stage disease by capturing the nuclei of nearly 100,000 cells, uncovering insights into cellular composition, transcriptional pathways, and gene expression associated with ischemic cardiomyopathy. Furthermore, Wang et al. (10) have utilized scRNA-seq to investigate the differences between the right and left ventricle, observing a significant number of differ-

entially expressed genes between the two chambers and characterizing the different functions of each chamber. This integrated approach of scRNA-seq and snRNA-seq has significantly advanced our comprehension of gene expression patterns in cardiac biology. These technologies offer deep insights into the cellular and molecular dynamics of the heart, paving the way for more targeted and effective treatments for heart diseases.

Single-Cell Epigenomics

Single-cell epigenomics has emerged as a powerful tool for elucidating the intricate landscape of DNA accessibility and epigenetic variability within individual cells. Epigenetic modifications, including histone modifications, play a pivotal role in governing chromatin structure, thereby influencing DNA accessibility and gene expression. Notably, Rotem et al. (24) advanced the field by pioneering a method that combines drop-based microfluidics, genomic barcoding, and next-generation sequencing, establishing the method for profiling chromatin at single-cell resolution, thereby revealing cell-to-cell variability.

One of the instrumental techniques in exploring DNA accessibility directly is ATAC-seq, which utilizes the Tn5 transposase to bind, fragment, and tag DNA sequences within accessible chromatin regions (1). The extension of this approach to single-cell resolution, known as scATAC-seq, has been achieved through various cell-sorting strategies. Buenrostro et al. (25) notably developed scATAC-seq uti-

lizing Tn5 tagmentation to map the accessible genome of individual cells, based on the CPW platform. Additionally, a droplet-based single-cell capturing approach was introduced, further expanding the capabilities of scATAC-seq (26). Furthermore, Zhang et al. (13) provide additional context on how single-cell epigenomics could be applied in the context of cardiovascular research. Single-cell epigenomic investigations such as scATAC-seq, single cell chromatin immunoprecipitation sequencing, and scDNA methylation-seq have contributed to our understanding of changes in accessible chromatin regions across various cell populations in response to injuries like myocardial infarction, offering valuable insights into the dynamic epigenetic responses within the heart.

Single-cell epigenomics, particularly through the utilization of techniques like scATAC-seq, has unveiled the intricate web of DNA accessibility and epigenetic modifications at unprecedented resolution. These advancements are poised to enhance our understanding of cardiac biology and hold promise for deciphering the epigenetic underpinnings of cardiovascular diseases.

Single-Cell Proteomics

Studying proteins at individual cell level, particularly focusing on their post-translational modifications and how they interact, is crucial for a deeper understanding of how cells function. Numerous studies that integrate multiple omics approaches tend to analyze proteins and mRNA together using selected antibody panels, which potentially overlooks new proteomic insights (27). In 2016, the Proximity Ligation Assay for RNA quantified over 40 targeted mRNAs and proteins simultaneously in 10,000 single cells across diverse cell types (28). A different method involved using a Proximity Extension Assay (PEA) to examine nearly 100 protein targets in single-cell lysates, also allowing for the simultaneous detection of RNA via real-time polymerase chain reaction (29). That same year, a holistic method that merged PEA with targeted amplification was able to identify both RNAs and proteins in individual cells using the Fluidigm C1 Platform (30).

A major leap forward in simultaneously analyzing the transcriptome and proteome came in 2017 with the introduction of Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq) (31). This technique combines multiplexed detection of protein markers with comprehensive transcriptome analysis in thousands of single cells. The RNA expression and protein sequencing assay also enables co-profiling by quantifying surface proteins with 82 antibodies and large-scale mRNAs within a

single protocol (32). Single-cell combinatorial indexed cytometry sequencing showcased the use of combinatorial indexing with splint oligonucleotides for the multiplexed analysis of over 150 surface proteins and mRNA expression in individual cells (33). By integrating sample multiplexing with droplet technology, the co-analysis of mRNA and proteins has been significantly optimized. Methods like RNA and immunodetection are among those that assess both intracellular and cell-surface proteins, facilitating the study of how cellular reactions are linked to external stimuli (34). Technologies such as ATAC with select antigen profiling by sequencing (ASAP-seq) and DOGMA-seq allow for the simultaneous profiling of chromatin accessibility, gene expression, and protein levels (35). Meanwhile, NEAT-seq offers a comprehensive view by co-profiling nuclear protein epitopes, chromatin accessibility, and the transcriptome in single cells, enhancing our understanding of the mechanisms behind gene regulation (35). For instances, Mimitou et al. (35) have conducted a two-stage data integration process on the ASAP-seq and CITE-seq datasets to maintain the biological impacts of the stimulation and residualize difference between the RNA and ATAC assays, suggesting that proteomic technologies can be used with single-cell transcriptomics in parallel in cardiovascular research.

Single-cell proteomics enhances cardiovascular research by dissecting individual cardiac cells and unraveling unique protein signatures and cellular heterogeneity. This precision reveals crucial insights into disease mechanisms, identifying specific protein alterations associated with cardiac disorders (36). By elucidating cell-specific responses and uncovering key molecular pathways, single-cell proteomics enhances our understanding of cardiovascular diseases, paving the way for personalized therapeutic strategies (37).

Single-Cell Multi-Omics

A critical challenge within scRNA-seq technology is the escalating costs incurred during the preparation of RNA-seq libraries from individual cells. The financial burden increases proportionally with the number of cells processed, which is a concern encountered by laboratories and research consortia. However, innovative multiplexing methods have emerged as a solution to alleviate this challenge by being more efficient and enhance throughput per cell. Multiplexing combinatorial indexing is comprised of two independent yet conceptually analogous methods, namely single-cell combinatorial indexing RNA sequencing and split pool ligation-based transcriptome sequencing, this technique uniquely labels transcriptomes through multiple rounds of cell

splitting and barcode indexing. As a result, it enables the analysis of up to ~2 million cells derived from over 60 embryos in a single run. Despite the augmented throughput obtained by combinatorial indexing, it may entail some compromises in sequencing depth, thereby limiting the ability to identify rare cell populations. Consequently, careful consideration of the balance between breadth and depth becomes necessary in experimental design.

Complementing the advancements in scRNA-seq, several other single-cell omics techniques are either in development or active use. These include scATAC-seq, DNA methylomics, and proteomics. scATAC-seq, for instance, offers the capability to simultaneously probe chromatin accessibility in tens of thousands of cells, thus unveiling regulatory landscapes across diverse biological contexts. Nevertheless, it is important to acknowledge that this technique necessitates additional steps for isolating single nuclei. Moreover, the integration of single-cell DNA methylomes with scRNA-seq or chromatin accessibility data aids in uncovering coordinated mechanisms of epigenetic and transcriptional regulation. Challenges persist within the realm of single-cell proteomics, particularly in achieving comparable sensitivity and throughput. Ongoing efforts to enhance detection methods and refine workflows are underway to address these limitations.

Additionally, spatial transcriptomics has emerged as a powerful technology that adds a new dimension to omics techniques, offering near single-cell resolution. Methods such as Slide-seq have enhanced spatial resolution, enabling high-dimensional structural analysis of tissues and organoid models. The advent of integrated spatial omics techniques, including cell village and chromatin accessibility mapping, holds the promise of facilitating high-dimensional multi-omics analyses, which can be traced back to individual cells. The field of single-cell multi-omics is rapidly evolving, with innovative solutions addressing the challenges posed by escalating costs and complexities associated with single-cell analyses.

Single-Cell Omics Technology Applications in iPSC-Based Cardiac Studies

Many scientists have introduced iPSC technology in cardiac and other organ research. The emergence of iPSC technology using the transcription factors OCT3/4, c-MYC, SOX2, and KLF4 (38) has significantly changed how biomedical studies are carried out. By introducing these four transcription factors that function in reprogramming a differentiated cell into a pluripotent cell, it is possible to induce iPSCs that share similarities with embryonic stem

cells which can proliferate continuously and differentiate into many sorts of cells such as CMs, endothelial cells, and fibroblasts. In addition, this technology can bridge the gap of data derived from species differences using conventional animal models, and the ethical problem of human embryonic stem cells (39).

iPSC technology is available to study cardiac biology. Also, researchers can model heart diseases using human iPSC (hiPSC) obtained from patients instead of animal models since iPSCs are genetically identical to the donors and linked with drug testing and screening based on iPSCs (40). Nowadays, cardiac research based on iPSC technology has been combined with single-cell transcriptome analysis to improve our knowledge of the human heart, in a manner of inducing specific cardiac cells and conducting scRNA-seq to each cell type (Table 1).

Single-Cell Analysis of iPSC in Basic Cardiac Research

The application of single-cell transcriptome analysis in iPSCs has revealed novel insights into the biology of the human heart, particularly with cardiac differentiation and human development. A notable study employed single-cell sequencing to explore gene expression patterns during hiPSC differentiation, leading to the discovery of the gene *HOPX* in regulating critical transcriptional networks associated with CM maturation during cardiac differentiation. This pioneering work in iPSC-derived cardiomyocytes (iPSC-CMs) using single-cell sequencing has provided valuable knowledge in the field (6). In CM subtypes, single-cell transcriptome analysis of iPSC-CMs demonstrated that the gene expression data of scRNA-seq did not completely match the specific CM subtypes exhibited by electrophysiology (41). Another study using scRNA-seq further focused on cell-type determination for cardiac differentiation of hiPSCs with the findings that variability of differentiated cells doesn't rely on the initial difference of hiPSCs despite the heterogeneity in gene expression of early hiPSC populations (42).

In cardiovascular research, iPSC-derived endothelial cells (iPSC-ECs) and iPSC-CMs are helpful (43). Large-scale single-cell sequencing of 5673 cells was performed during differentiation, thereby identifying four distinct populations of iPSC-ECs characterized by robust expression of four different membrane coding genes, *CLDN5* (claudin-5), *APLNR* (apelin receptor), *GJA5* (gap junction $\alpha 5$), and *ESM1* (EC-specific molecule 1). Robust expression of each gene leads to metabolically active iPSC-ECs, inflammation-responsive iPSC-ECs, arterial iPSC-ECs, and activated iPSC-

Table 1. Selected hiPSC-based cardiovascular research with single-cell omics technologies

Species	scRNA-seq method	Pluripotent cell type	Disease	Cell number	Finding	Publishing date	Data availability	Ref
Human	10X Genomics	hiPSC-CM	Healthy	17,599	Identification of time points for specifying the various cell types for differentiation	2022	GSE198729	(42)
Human	10X Genomics/ IFC system	hESC- and hiPSC-derived cells	Healthy	43,168	Identification of fundamental mechanisms underlying heart development and differentiation of hiPSC-CMs at single-cell resolution	2018	GSE97080	(6)
Human	10X Genomics	Patient iPSC-CMs	Oxidative stress (congenital heart disease)	8,094	mPTP inhibition & TUDCA, potential therapeutic targets for suppressing endoplasmic reticulum (ER) stress	2022	GSE146341	(21)
Human	10X Genomics	hiPSC and derivatives	TBX5 haploinsufficiency (congenital heart disease factor)	55,782	Discovery of sensitivity to TBX5 dosage in heterogeneous subsets of iPSC-CMs at single-cell resolution	2021	GSE137876	(52)
Human	10X Genomics	hiPSC-derived cardiac organoid (hiPSC-CMs, fibroblasts, and endothelial cells)	Healthy	N/A	Effects of immunosuppressive drugs (tacrolimus and sirolimus) using iPSC-derived cardiac organoid and scRNA-seq	2022	GSE188211	(62)
Human	scRNA-seq	Patient-derived iPSC-CMs	Hypoplastic left heart syndrome	9,899	Observation of impaired contractility of HLHS iPSC-CMs with associated changes in gene expression	2020	GSE146763	(53)
Human	10X Genomics	Patient-derived iPSC-endothelial cells	Hypoplastic left heart syndrome	2,383	Identification of robust endocardial functional defects and aberrant endocardium-myocardium crosstalk in HLHS	2020	GSE138979	(54)
Human	10X Genomics	Patient-derived iPSC-CMs and engineered cardiac tissues	Pulmonary atresia with intact ventricular septum (PA/IVS)	25,059	Identification of relationships with cardiac developmental trajectory during the fetal stage that leads to this cardiac anomaly	2020	GSE157157	(55)
Human	10X Genomics	hiPSC-endothelial cells	Healthy	5,673	Identification of iPSC-EC subpopulations and their biological function	2018	GSE116555	(44)
Human	10X Genomics	Co-cultured hiPSC-CMs and hiPSC-ECs	Healthy	4,000	Identification of interactions and transcriptomic changes induced by endothelial cell-CM crosstalk	2021	GSE150741	(45)

hiPSC: human induced pluripotent stem cell, scRNA-seq: single-cell RNA sequencing, Ref: reference, hiPSC-CM: hiPSC-derived cardiomyocyte, IFC: integrated fluidic circuit, hESC: human embryonic stem cell, iPSC: induced pluripotent stem cell, iPSC-CMs: iPSC-derived cardiomyocytes, HLHS: hypoplastic left heart syndrome, iPSC-EC: iPSC-derived endothelial cell.

ECs that are related to angiogenesis and cellular response, respectively (44). Furthermore, single-cell transcriptome analysis of co-cultured hiPSC-ECs with iPSC-CMs highlighted the significance of intercellular interactions between these cell types. The findings revealed that hiPSC-CM is crucial in promoting gene expressions associated with cardiac differentiation and development (45).

Single-Cell Analysis of iPSC in Cardiac Disease Modeling

The use of hiPSCs, particularly derived from patients, has proven invaluable in creating cellular models for various cardiac diseases such as Long QT syndrome (46), Timothy syndrome (47), dilated cardiomyopathy (48), and hypertrophic cardiomyopathy (49). These disease models play a crucial role in unraveling the underlying mechanisms of cardiac diseases and are essential for advancing our understanding of these conditions. Nevertheless, the heterogeneity of iPSCs still has been elusive. In this circumstance, applying single-cell transcriptomic analysis has deepened our understanding of the mechanism underlying cardiac diseases. iPSC-CMs from patients with genetic mutations to the Lamin A/C gene were examined with scRNA-seq, demonstrating the utility of integrating iPSC technology with single-cell technology in studying cardiac diseases (50). Another study modeled a dominant pathogenic mutation of the TGFBR1 gene and revealed that this mutation caused cell type-specific defects restricted to smooth muscle cells from cardiovascular progenitor cells (51). Also, modeling human TBX5 haploinsufficiency based on iPSCs coupled with single-cell analysis explained the impact of TBX5 dosage in heterogeneous subtypes of iPSC-CMs (52).

In addition to the cardiac diseases mentioned above, iPSCs have also been utilized to model various congenital heart diseases (CHDs). ScRNA-seq was applied to iPSC-CMs from hypoplastic left heart syndrome (HLHS) patients with RV failure and exhibited transcriptional differences in G1-phase between HLHS and control cells (53). Another study about heart failure (HF) associated with HLHS demonstrated that early HF is related to mitochondrial dysfunction along with endoplasmic reticulum stress using single-cell transcriptome analysis (21). Also, hiPSC-ECs on which scRNA-seq was performed were utilized to identify the defective endocardial population in HLHS patients, finally uncovering the contribution of developmentally intrinsic endothelial abnormalities to HLHS pathology (54).

Moreover, integrating scRNA-seq and three-dimensional (3D) modeling with iPSC demonstrated that intrinsic abnormalities in several biological processes disrupted the dif-

ferentiation of early cardiac progenitor lineages, leading to abnormal differentiation and maturation of CM subpopulation in the HLHS (54). Modeling of the PLN R14del cardiomyopathy with hiPSC-CMs in both 2-dimensional cultures and 3-dimensional engineered heart tissues revealed that unfolded protein response (UPR) is induced due to mutation, suggesting regulation of UPR as the potential use therapeutically. By performing scRNA-seq on hiPSC-CMs and engineered heart tissues, which modeled pulmonary atresia with the intact ventricular septum, researchers identified transcriptomic differences resulting in developmental abnormalities and contractile defects in hypoplastic right heart syndrome (55).

Single-Cell Omics Application in Drug Discovery

iPSC technology has been used as an essential means to research drugs for drug screening and cardiotoxicity testing in cardiovascular studies. Before the common use of hiPSC and its derivatives like iPSC-CMs, animal models and cardiac ion channels have been generally utilized to predict unknown cardiotoxicity. However, animal models were high-priced and low throughput, not to mention interspecies variation. Cardiac ion channels had limitations due to non-cardiac cells which have different properties from CMs (56). The first hiPSC-CMs showed a promising *in vitro* model for drug screening and analyses by identifying their response to various cardiac drugs (57). Further, iPSC has been widely used with the establishment of the Comprehensive *in vitro* Proarrhythmia Assay, a standardized evaluation of cardiac safety that aids in estimating cardiac safety and arrhythmogenesis (56). Through hiPSC-CMs, 3D models, drug-induced changes and cardiotoxicity have been examined (58-61).

Combined with single-cell sequencing, investigators can overcome the shortcomings of looking at average signal expression from the conventional approaches. Now, researchers can examine the effect of drugs on the human heart at a single-cell resolution. Most recently, scRNA-seq was performed to investigate the cardiovascular effects of two immunosuppressive drugs—tacrolimus and sirolimus—using hiPSC-derived cardiac organoids after comparing phenotypic differences concerning drugs (62). In this experiment, single-cell sequencing played a central role in recognizing cell type-specific responses to immunosuppression drugs which could explain some of the physiological differences observed clinically. Also, scRNA-seq contributed to presenting *in vitro* platforms as a powerful tool for drug research by identifying disease-specific genes, pathways, and features within single cells and treating associated

compounds, which can result in novel drug discovery (51, 63, 64). Accordingly, with further advances in single-cell platforms and their collaboration with iPSC technology, single-cell technologies will be instrumental in cardiac research.

Single-Cell Omics Application in Other Areas of Cardiac Research

Recent technological advances have made it possible to conduct a comprehensive analysis of the cellular and molecular landscape at the single-cell level more efficiently. scRNA-seq and scATAC-seq provide an unbiased assessment of transcriptomes and epigenomes in heterogeneous tissues. Researchers have discovered not only previously unknown cell populations but also dynamic changes in cells and interactions between cells within different tissues. The technologies mentioned above have been used to make reference maps, like the Human Cell Atlas, including both healthy organisms, normal development, and diseases. Single-cell transcriptomics has shed light on the diversity of vascular CMs. Hnatiuk et al. (40) argued that the transcriptome and proteome of the single-cell atlas should be utilized to better comprehend such variety as the cell population in hiPSC-CM culture and *in vivo*. Using Tabula Muris, a mouse scRNA-seq atlas, Zhang et al. (63) identified a specific gene of human cardiac fibroblast.

Moreover, scRNA-seq has facilitated the identification of disease-causing gene expression and mutations at the individual cell level, offering unprecedented insights into cell developmental trajectories. Hulin et al. (65) utilized droplet-based transcriptome sequencing to reveal that endothelial and immune cell subsets maintain relative stability during aortic and mitral valve development in neonatal d7 (primitive valves) and d30 (mature valves) mice, while interstitial cell subsets undergo substantial changes. This study represents the inaugural exploration of cellular diversity during heart valve remodeling, presenting novel avenues for investigating valve homeostasis and molecular mechanisms underlying valve diseases. scRNA-seq's unique technical advantages have unveiled cell-to-cell heterogeneity in disease and drug responses, fostering the development of cardiac precision medicine and effective therapeutic strategies for cardiovascular diseases (66).

The future heart atlas from iPSC data can be used as a database for disease research and drug response research, as well as revealing and estimating genetic changes in iPSC differentiation. Cellular communication and interactions between cells and tissues are critical for regulating biological functions. Various mechanisms mediate

these interactions, including ligand-receptor interactions, soluble factors like growth factors and cytokines, and mechanical forces. Understanding the regulatory mechanisms in complex tissues requires spatial information. Spatial transcriptomics, a revolutionary profiling method compensating for the spatial information loss in scRNA-seq, provides valuable insights into molecular mechanisms within specific regions (67-69). Researchers have evaluated the anatomical context of TBX5-dependent genes in iPSC-CMs, which provides insights into their expression during human cardiac morphogenesis and their relevance to heart outflow tract defects (39) and CHDs (52).

Conclusions

Cardiovascular disease is one of the leading causes of death worldwide, making cardiac research essential to understanding heart development, physiology, and pathology. Both iPSCs and single-cell multi-omics are recent technologies that have significantly contributed to the rapid growth of the cardiovascular field in fundamental research, disease modeling, and the discovery of novel drugs. By providing patient-specific cell types for disease modeling and drug screening, iPSCs are expected to usher in a new era of personalized medicine.

However, there are limitations to overcome in cardiac iPSC research. *In vivo* cardiac development is a sophisticated biological process that requires complex intercellular interactions with 3-dimensional spatiotemporal cues to guide cell signaling and migration, which cannot be replicated in hiPSC-CMs in a dish (55). This could be caused by a lack of protocols to recapitulate specific intracardiac sites such as endocardial function *in vivo* (54). Furthermore, despite the advancements in single-cell omics analysis, there still remain limitations in the scRNA-seq technique of sparse information with variable values and more background noise compared to the bulk-RNA seq. Along with the ongoing efforts to identify the source of noise and data variability, there needs to be continuous research to improve the quality of data processing and background noise removal methods. In addition to these technical constraints, implementation necessitates standardized protocols for sample preparation and data analysis, as well as physician-friendly interfaces and software.

Despite these limitations, applying single-cell omics technologies in iPSC-based cardiovascular research is the best choice for future biomedical studies and drug development. Single-cell analysis not only unravels the lack of evidence for *in vivo* endocardial function reproduction but also mitigates the obscurity of individual patients' genetic

traits regardless of its complexity, by virtue of its potential to analyze cell sub-populations in detail at multicomplex levels (70). Furthermore, single-cell technologies complemented by the spatial transcriptomics technology that can reveal the spatial position of each cell could be used to discover characteristics of cells within its specific niche and cell-to-cell interactions. In this context, the necessity of single-cell technology for further cardiovascular research is indeed accentuated. With the continued improvement of scRNA-seq pipelines, the decreasing costs of sequencing, and the advancement of data-driven precision medicine, integration of translational single-cell applications will continue to grow. They will more than likely redefine how patients are treated in the future (71).

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Potential Conflict of Interest

There is no potential conflict to declare.

Authors' Contribution

Conceptualization: SR, KOJ. Data curation: HK, SC, SHC. Formal analysis: HK, SC, HH. Funding acquisition: SR. Methodology: HK, YL, HH. Project administration: SR. Resources: SR. Supervision: SR, KOJ. Validation: SHC, YL, DK, KOJ. Visualization: HJK, HH, YL. Writing – original draft: HK, SC, HH, YL. Writing – review and editing: HK, SC, DK, SR.

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